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ORIGINAL ARTICLE *Reproductive endocrinology*

GnRHa trigger and individualized luteal phase hCG support according to ovarian response to stimulation: two prospective randomized controlled multi-centre studies in IVF patients

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STUDY QUESTION: Does a GnRH agonist (GnRHa) trigger followed by a bolus of 1.500 IU hCG in a group of patients at risk of ovarian hyper-stimulation syndrome (OHSS) reduce the OHSS incidence compared with hCG trigger?

SUMMARY ANSWER: A GnRHa trigger followed by early luteal hCG support with one bolus of 1.500 IU hCG appears to reduce OHSS in patients at risk of OHSS; however, in a low-risk group a second bolus of 1.500 IU hCG induced two cases of late onset OHSS.

WHAT IS KNOWN ALREADY: A GnRHa trigger is an alternative to hCG in GnRH antagonist co-treated cycles.

STUDY DESIGN, SIZE, DURATION: Two RCTs were performed in four Danish IVF units. A total of 446 patients were assessed for eligibility and 390 patients were enrolled in the study from January 2009 until December 2011. The primary outcome of the study was OHSS incidence in the group at risk of OHSS.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Patients received a fixed dose of recombinant human FSH for the first 4 days. On the day of triggering, patients were assessed for their risk of OHSS based on the total number of follicles ≥11 mm diameter, and were classified as being at risk of OHSS when the total number of follicles ≥11 mm was between 15 and 25 and at low risk of OHSS when the total number of follicles ≥11 mm was ≤14. Two separate randomization lists were used for each of the OHSS risk groups. Women at risk of OHSS were allocated (RCT 1) to either: Group A (n = 60), ovulation triggering with a bolus of 0.5 mg buserelin (GnRHa) s.c. followed by a single bolus of 1.500 IU hCG s.c. after the oocyte retrieval—or: Group B (n = 58): 5.000 IU hCG. Similarly, women at low risk of OHSS were allocated (RCT 2) to receive either: Group C (n = 125), a bolus of 0.5 mg buserelin s.c., followed by a bolus of 1.500 IU hCG s.c. after oocyte retrieval and a second bolus of 1.500 IU hCG on the day of oocyte retrieval +5—or: Group D (n = 141), 5.000 IU hCG. Groups C and D were included in order to obtain preliminary data.

MAIN RESULTS AND THE ROLE OF CHANCE: In women at risk of OHSS (RCT 1) (15−25 follicles) no OHSS case was seen in Group A (GnRHa trigger and one bolus of 1.500 IU hCG), whereas two cases of moderate late-onset OHSS occurred in group B (3.4%), (P = 0.24). In contrast, in women at a low risk of OHSS (RCT 2) (≤14 follicles) two cases of late-onset OHSS occurred in Group C (GnRHa trigger and two boluses of 1.500 IU hCG), whereas no OHSS case was encountered in Group D (P = 0.22).

LIMITATIONS, REASONS FOR CAUTION: Although the first RCT was powered to include 168 patients at risk of OHSS (15−25 follicles ≥11 mm) randomized to either GnRHa trigger or hCG trigger, the trial was prematurely discontinued when a total of 118 patients at risk of OHSS were randomized. In addition the second RCT in the OHSS low-risk group was designed as a feasibility study to assess the incidence of OHSS after GnRHa trigger and dual hCG administration versus 5.000 IU hCG. No power calculation was performed for this trial. In addition, there was a lack of blinding in the RCTs.

WIDER IMPLICATIONS OF THE FINDINGS: Although a non-significant result, one bolus of 1.500 IU hCG after GnRHa trigger tended to reduce the OHSS rate in patients with 15–25 follicles ≥11 mm as well as secure the ongoing pregnancy rate. In contrast, in patients at low risk of OHSS the administration of two boluses of 1.500 IU hCG after GnRHa trigger should be avoided as it may induce OHSS.

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Key words: GnRH agonist trigger / GnRH antagonist / hCG / ovarian hyperstimulation syndrome / IVF

Introduction

A bolus of 5.000–10.000 IU hCG has been successfully used for decades in assisted reproduction technology (ART) as a substitute for the endogenous LH surge to induce final oocyte maturation prior to oocyte retrieval due to the fact that LH and hCG both bind to and activate the LH/hCG receptor (Kessler et al., 1979). The half-life of hCG is significantly longer (days) than that of endogenous LH (hours) (Hoff et al., 1983; Weissman et al., 1996) and thus, a bolus of hCG leads to a prolonged luteotropic effect which in combination with the formation of multiple corpora lutea (CL) may lead to the development of ovarian hyperstimulation syndrome (OHSS) (Haning et al., 1985).

Importantly, the natural mid-cycle surge of gonadotrophins also includes an FSH surge. Although the presence of a mid-cycle surge of FSH is not mandatory, it is known to promote nuclear maturation as well as LH receptor formation on the luteinizing granulosa cells (Strickland and Beers, 1976; Eppig, 1979; Zelinski-Wooten et al., 1995; Yding et al., 1999; Yding, 2002).

In IVF/ICSI patients co-treated with a GnRH antagonist, a bolus of GnRH agonist (GnRHa) may be administered as an alternative to hCG for final oocyte maturation. This ovulation trigger concept was previously shown to stimulate effectively final oocyte maturation and ovulation, as GnRHa displaces the GnRH antagonist from the GnRH receptor, inducing an initial activation (flare-up) of LH and FSH, similar to that of the natural cycle prior to down-regulation of the receptor (Gonen et al., 1990; Itskovitz et al., 1991). However, the profile and duration of the GnRHa-induced surge of gonadotrophins are significantly different and shorter than those of the natural cycle (Hoff et al., 1983), contrasted by the sustained LH-like activity induced by hCG trigger (Yding et al., 1993). This leads to a luteal phase insufficiency with very low LH levels after the GnRHa trigger (Beckers et al., 2003; Humaidan et al., 2005, 2012) and malfunctioning CL (Casper and Yen, 1979; Sugino et al., 2000; Licht et al., 2001; Wang et al., 2002).

Previously, the use of a GnRHa trigger in IVF/ICSI cycles followed by only a standard luteal phase support resulted in implantation failure and a high early pregnancy loss rate (Beckers et al., 2003; Humaidan et al., 2005, 2010, 2011b; Andersen et al., 2006). However, modifying the luteal phase support by adding a bolus of 1.500 IU hCG on the day of oocyte retrieval significantly reduced the early pregnancy loss rate and tended to increase the ongoing pregnancy rate when compared with hCG trigger (Humaidan et al., 2006, 2010). Although non-significant, the difference in reproductive outcome was in favour of hCG trigger by 7%, and we suggested further studies to optimize the luteal phase support after GnRHa trigger while maintaining a low risk of OHSS.

Importantly, no OHSS was seen after GnRHa trigger despite supplementation with a small bolus of hCG, contrasted by an OHSS rate of 2% seen after hCG trigger (Humaidan et al., 2010).

The aim of the present study was to answer two different questions. First, whether GnRHa trigger followed by one bolus of 1.500 IU hCG decreases the OHSS rate in women at risk of OHSS (15–25 follicles ≥11 mm) compared with conventional hCG trigger. Secondly, whether the administration of an additional bolus of 1.500 IU hCG during the early luteal phase in the OHSS low-risk patient (≤14 follicles of ≥11 mm) can be considered a safe option. A cut-off value of >14 follicles ≥11 mm was set to distinguish between women at risk of OHSS and patients at low risk of OHSS (Papanikolaou et al., 2006). Patients with >25 follicles were excluded from the study to prevent possible randomization to hCG trigger.

The primary outcome of the study was the OHSS incidence in the group at risk of OHSS. The secondary outcome measures were the OHSS rate in the OHSS low-risk group and the reproductive outcome after GnRHa trigger versus hCG trigger.

Materials and Methods

The study was approved by the Ethics Committee of Viborg County—Project number: VN-20060036MCH. Clin Trial.gov number: NCT00627406. Written informed consent was obtained from all participants.

Patients' selection process

Patients fulfilling the following inclusion criteria were considered eligible to participate in the trial: (i) female age >25 and <40 years; either (ii) normal menstrual cycles of 25–34 days or (iii) oligomenorrhea/amenorrhea or (iv) polycystic ovary syndrome, defined according to Rotterdam criteria (2004), (v) BMI > 18 and <35 kg/m^2 and (vi) absence of uterine abnormalities. Patients were excluded from the study if they had hypothalamic dysfunction, diabetes, epilepsy, liver, renal or heart disease or metabolic disorders. Ovarian reserve testing was not used as an inclusion/exclusion criterion.

Hormonal treatment

Ovarian stimulation was initiated with recombinant human FSH (Puregon; Organon, Skovlunde, Denmark) from cycle Day 2 or 3 and continued until the day of ovulation induction. A fixed dose of recombinant human FSH was used: either 150 or 200 IU per day for the first 4 days, according to the antral follicle count on cycle day 2–3. After 4 days, doses were adjusted according to ovarian response. A fixed GnRH antagonist protocol was used, commencing from stimulation Day 5 in the morning. From this day onwards a bolus of 0.25 mg/day of the GnRH antagonist ganirelix (Orgalutran;

Organon, Skovlunde, Denmark) was administered up to and including the day of ovulation induction.

Ovulation trigger

As soon as two follicles had reached a diameter of 17 mm, all patients who met the eligibility criteria and had provided their written informed consent were randomized.

Two different randomization lists were available depending on the number of follicles seen on transvaginal ultrasound examination on the final day of ovarian stimulation: one for patients with >14 follicles ≥11 mm diameter (at risk of OHSS) and one for patients with ≤14 follicles of ≥11 mm (OHSS low-risk group).

Randomization

The randomization was performed by a study nurse, using computer-generated random numbers in sealed, unlabelled envelopes, each containing a unique study number. Each patient contributed with one cycle only. Doctors, participants and nurses administering the interventions were not blinded to group assignment. As previously mentioned, patients with >25 follicles ≥11 mm on the day of ovulation trigger were excluded from randomization.

Randomization of patients at risk of OHSS

The group at risk of OHSS was randomized to two groups, either: Group A, triggering of final oocyte maturation with a bolus of 0.5 mg buserelin (GnRHa) s.c. (Suprefact; Hoechst, Hoersholm, Denmark), followed by a single bolus of 1.500 IU hCG IU, s.c. (Pregnyl; Organon, Skovlunde, Denmark) after the oocyte retrieval—or: Group B, 5.000 IU hCG (Pregnyl; Organon, Skovlunde, Denmark).

Randomization of patients at low risk of OHSS

The OHSS low-risk group was randomized to triggering of final oocyte maturation with either: Group C, a bolus of 0.5 mg buserelin s.c. (Suprefact; Hoechst, Hoersholm, Denmark), followed by a bolus of 1.500 IU hCG, s.c. (Pregnyl; Organon, Skovlunde, Denmark) after oocyte retrieval and an additional bolus of 1.500 IU hCG on the day of oocyte retrieval +5—or: Group D, 5.000 IU hCG (Pregnyl; Organon, Skovlunde, Denmark).

Oocyte retrieval, embryo transfer and luteal phase support

All patients underwent oocyte retrieval 34 h after trigger. A maximum of two embryos were transferred on Day 2 or 3 after retrieval, following national criteria of single embryo transfer. For luteal phase support all patients received micronized progesterone vaginally, 90 mg twice daily (Crinone; Serono Nordic, Copenhagen, Denmark) and estradiol (E_2) 4 mg a day per os (Estrofem; Novo Nordisk, Copenhagen, Denmark), commencing on the day following the oocyte retrieval and continuing until 7 weeks of gestation.

Reproductive outcome

A biochemical pregnancy was defined by a plasma β-hCG >10 IU/L on Day 12 after embryo transfer. A clinical pregnancy was defined as an intrauterine gestational sac with a heartbeat 3 weeks after a positive hCG test. An ongoing pregnancy was defined as a viable pregnancy at Week 11 of pregnancy.

Blood samples and hormone assays

Blood sampling was performed: on stimulation Day 1 (S1), on the day of ovulation induction, on the day of oocyte retrieval and 7 and 14 days after oocyte retrieval. Sera were frozen at −20°C for subsequent analysis of E_2, FSH, LH, progesterone and hCG. LH and FSH were measured by time-resolved immunofluorometric assay, the AutoDelfia spec.kit (Wallac Oy, Turku, Finland). E_2, progesterone and hCG were measured according to manufacturer's instructions using a commercially available radioimmunoassay kit intended for measurements in serum (DSL-4200; Diagnostic System Laboratories, Texas, USA). All assays were intended for measurements in serum samples.

Outcome measures

The primary outcome of the study was the OHSS rate in the groups at risk of OHSS. The secondary outcome measures were the OHSS rate in the OHSS low-risk groups and the reproductive outcome after GnRHa trigger versus hCG trigger.

Classification of OHSS

Following the classification of Navot et al. (1992), moderate OHSS was defined as abdominal distension and discomfort, nausea with or without vomiting, ultrasound evidence of ascites, ovarian size of 8–12 cm, haematocrit <45% and weight gain <2 kg.

Severe OHSS was defined as variable ovarian enlargement, ascites with or without hydrothorax, haematocrit >45%, weight gain >2 kg, white blood cell count >15.000, oliguria, creatinine of 1.0–1.5, creatinine clearance of >50 ml/min, liver dysfunction and oedema anarsaca.

Sample size

Sample size calculation was performed for the primary outcome of the study, the OHSS incidence in women at risk of OHSS (>14 follicles ≥11 mm). We calculated that a total of 168 patients are needed (84 in each arm) to detect an 11% difference in the incidence of moderate to severe OHSS in the OHSS risk group between treatment arms (1% incidence in the GnRHa triggering group versus 12% in the hCG group). The OHSS incidence of 12% in the hCG group was derived from observations by Lainas et al. (2007). This sample size was calculated using a two-sided Fisher's exact test with a power of 80% and a level of significance was set at $P < 0.05$.

As the enrolment of patients and written informed consent took place on the first day of consultation, prior to randomization (on the day of triggering final oocyte maturation), we were unable to a priori estimate the number of patients needed to be enrolled in order to reach the sample size of 168 patients with >14 follicles >11 mm on the day of final oocyte maturation, as calculated by the power analysis for the primary outcome of the study. However, based on a previous randomized study by our group, in which approximately one-third of the patients had developed >14 follicles (Humaidan et al., 2010), we estimated that we needed to enrol 600 patients in order to reach at least 168 patients with >14 follicles to be randomized, as calculated by the two-sided Fisher's exact test.

Patients having ≤14 follicles on the day of triggering, who already signed their informed consent to participate in the trial, were also randomized, using a separate randomization list, to receive either a bolus of 0.5 mg buserelin s.c. followed by a bolus of 1.500 IU hCG s.c. after the oocyte retrieval and an additional bolus of 1.500 IU hCG on the day of oocyte retrieval +5—or 5.000 IU hCG. For this specific outcome, no sample size calculation was performed due to the fact that dual administration of 1500 hCG during the early luteal phase has not been tested in a clinical setting. Thus, patients with ≤14 follicles who signed informed consent were randomly allocated to the two treatment groups in order to retrieve preliminary data for future studies.

Statistical methods

Categorical values were analysed by the use of the chi square or Fisher's exact test. In addition relative risks and 95% confidence interval were calculated

between compared groups to assess differences. For continuous variables analysis was performed by use of the independent *t*-test or Mann–Whitney *U*-test, depending on the normality of the results. Normality of the distribution was assessed by use of the Shapiro–Wilk test. All values were two-tailed with a level of significance set at $P < 0.05$. Analyses were performed using the Statistical Package for the Social Sciences 20 software.

Results

Randomization

Overall 446 patients were assessed for eligibility and 390 patients were enrolled in the study. Six patients were not randomized due to an inadequate ovarian response, leaving a total of 384 patients for randomization. Unfortunately the study had to be discontinued before the estimated sample size had been obtained due to the death of one of the local principal investigators and job rotations among other investigators.

Patients at risk of OHSS

A total of 118 patients were classified as patients at risk of OHSS with >14 follicles ≥ 11 mm on the day of ovulation induction. Within the group at risk of OHSS, 60 patients were randomized to trigger with GnRHa, followed by one bolus of 1.500 IU hCG on the day of oocyte retrieval, and 58 patients to trigger with 5.000 IU hCG (Fig. 1).

Patients at low risk of OHSS

A total of 266 patients were classified as OHSS low-risk patients with 14 or less follicles of a diameter ≥ 11 mm on the day of trigger. Within this group, 125 patients were randomized to GnRHa trigger followed by one bolus of 1.500 IU hCG on the day of oocyte retrieval and an additional bolus on the day of oocyte retrieval +5, whereas 141 patients were randomized to 5.000 IU hCG trigger (Fig. 1).

Cancellations

Embryo transfer was cancelled in 23 patients in the GnRHa groups and in 26 patients in the hCG groups due to either total fertilization failure or poor embryo development. No empty follicle syndrome case was encountered in either GnRHa-triggered or hCG-triggered groups.

Patient characteristics and stimulation

The groups at risk of OHSS (A and B) and the OHSS low-risk groups (C and D) were compared pair-wise and no significant differences regarding demographic data were seen between groups. The majority (98%) of participants were Caucasians (Table I).

The total rFSH used and the duration of stimulation for the GnRHa and hCG groups, respectively, did not differ significantly (Table II).

The distributions of ICSI and IVF cycles, respectively, were: 30 and 30 (Group A), 23 and 35 (Group B), 59 and 66 (Group C) and 62 and 79 (Group D) (Table I). Comparable numbers of Day 2 and Day 3 transfers were performed in both groups (data not shown).

OHSS, groups at risk of OHSS A and B (>14 follicles)

No OHSS case was seen in Group A (GnRHa trigger and one bolus of 1.500 IU hCG) versus two cases of moderate late-onset OHSS occurred in group B (hCG trigger) (3.4%), ($P = 0.24$). Both patients were treated on an outpatient basis with intravenous rehydration (Table III).

OHSS, groups at low risk of OHSS C and D (≤ 14 follicles)

In group C (GnRHa trigger and two boluses of 1.500 IU hCG) two cases of severe late-onset OHSS occurred (1.6%), ($P = 0.22$ versus group D) and both were treated on an outpatient basis. The first case was a patient with a total of 11 follicles and 6 oocytes retrieved. Shortly after a positive pregnancy test she contacted the department due to pelvic distension and nausea. The other case occurred in a patient with 13 follicles and 13 oocytes retrieved, presenting herself 2 days before the pregnancy test with abdominal distension, nausea and breathing difficulties. In group D (hCG trigger) no OHSS case occurred. (Table III).

Oocytes and embryos, groups at risk of OHSS A and B (>14 follicles)

A similar number of oocytes was retrieved in Group A and Group B ($P = 0.93$), respectively. No differences between group A and B (IVF and ICSI) were seen regarding the percentage of transferable embryos ($P = 0.88$) (Table II). The mean (range) number of embryos transferred was 1.2 (1−2) versus 1.2 (1−2) ($P = 0.99$) and did not differ between groups A and B, respectively (Table III); however, a significant difference in the rate of transfer was seen in favour of hCG trigger ($P = 0.02$) (Table III).

Oocytes and embryos, OHSS low-risk groups C and D (≤ 14 follicles)

In groups C and D a similar number of oocytes were retrieved ($P = 0.21$). No differences between group C and D (IVF and ICSI) were seen regarding the percentage of transferable embryos ($P = 0.51$), (Table II). The mean (range) number of embryos transferred 1.3 (1−2) versus 1.3 (1−2) ($P = 0.98$) did not differ between groups C and D, respectively (Table III).

Reproductive outcome, groups at risk of OHSS, A and B (>14 follicles)

No significant difference was seen regarding the implantation rate between groups A and B ($P = 0.46$), respectively. No significant differences were seen regarding positive pregnancy tests per embryo transfer, ($P = 0.23$), clinical pregnancy rate per randomized patient ($P = 0.51$), and ongoing pregnancy rate per randomized patient ($P = 0.76$), respectively. No significant difference was seen regarding early pregnancy loss ($P = 0.78$) between groups A and B, respectively (Table III).
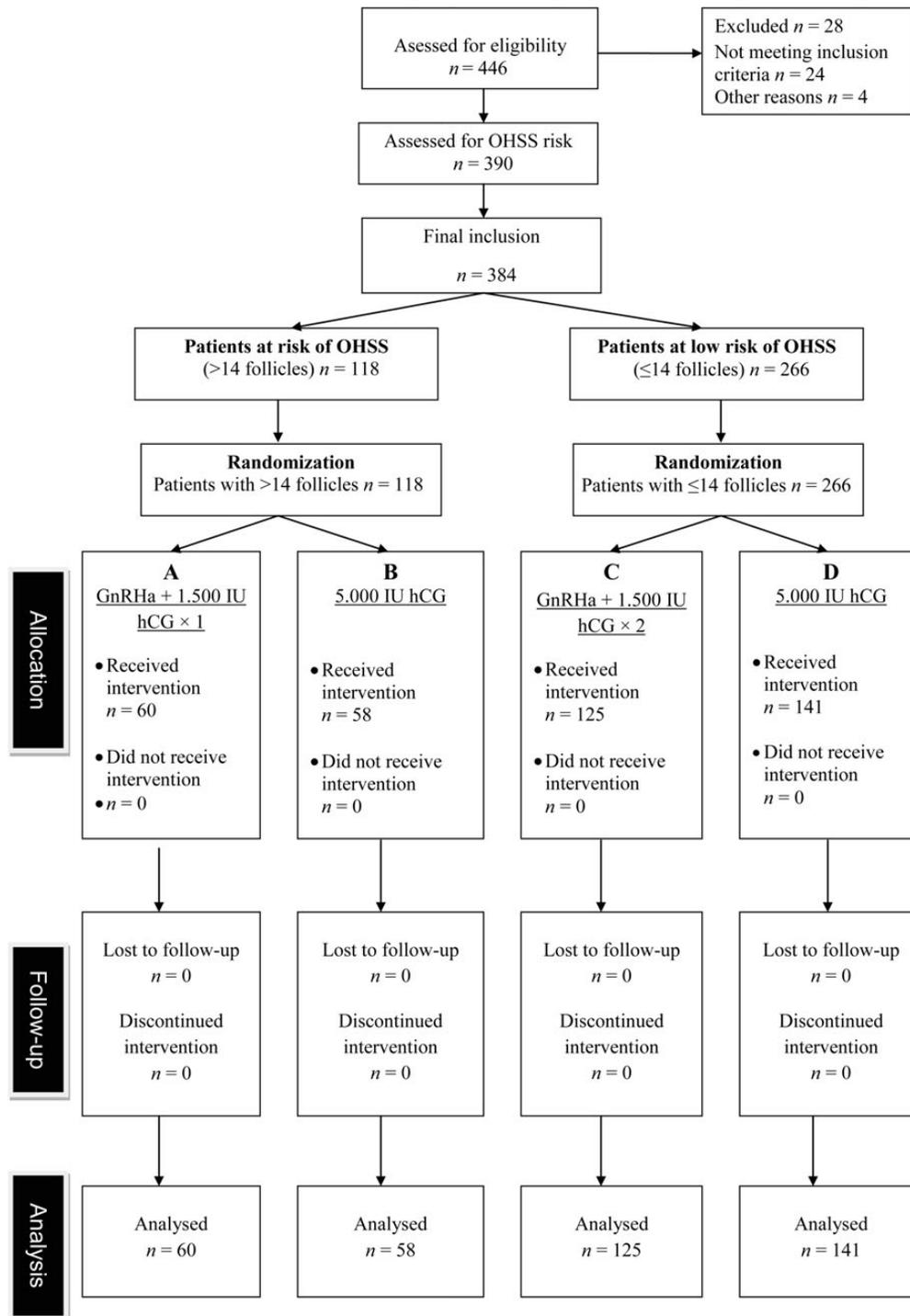
Figure 1 Flow chart for the study of GnRHa trigger and individualized luteal phase hCG support according to ovarian response/risk of OHSS, as carried out in two RCTs involving patients undergoing IVF/ICSI.

Reproductive outcome, OHSS low-risk groups C and D (≤14 follicles)

No significant difference was seen regarding implantation rate between groups C and D (*P* = 0.29), respectively. No significant differences were seen regarding positive pregnancy tests per embryo transfer (*P* = 0.25), clinical pregnancy rate per randomized patient (*P* = 0.28) and ongoing pregnancy rate per randomized patient (*P* = 0.45), respectively. No significant difference was seen regarding early pregnancy loss (*P* = 0.25), between groups C and D, respectively (Table III).

Table I Demographic data for women in the GnRHa versus hCG trigger groups.

	>14 follicles		P-value A versus B	≤14 follicles		P-value C versus D
	Group A: GnRHa trigger + 1.500 hCG (n = 60)	Group B: hCG trigger (n = 58)		Group C: GnRHa trigger + 1.500 hCG × 2 (n = 125)	Group D: hCG trigger (n = 141)	
Age, y [mean(SD)]	28.5 (5.2)	29.8 (3.8)	0.26	30.8 (3.9)	31.5 (3.7)	0.16
BMI (kg/m^2) [mean(SD)]	23.8 (2.9)	24.0 (3.7)	0.74	24.9 (3.9)	24.6 (3.6)	0.34
IVF, n (%)	30 (50.0)	35 (60.3)	0.71	66 (52.8)	79 (56.0)	0.60
ICSI, n (%)	30 (50.0)	23 (39.7)		59 (47.2)	62 (44.0)	
Infertility cause (%)						
Male	51.7	41.4	0.25	44.8	41.8	0.37
PCO	23.3	32.8		12.8	14.2	
Tubal	10.0	6.9		16.8	17.0	
Endometriosis	0	1.7		3.2	2.1	
Unexplained	15.0	17.2		22.4	24.8	

PCO, polycystic ovary.

Table II Results of ovarian stimulation, oocytes retrieved and embryos obtained for women in the two RCTs.

	>14 follicles		P-value A versus B	≤14 follicles		P-value C versus D
	Group A: GnRHa trigger + 1.500 hCG (n = 60)	Group B: hCG trigger (n = 58)		Group C: GnRHa trigger + 1.500 hCG × 2 (n = 125)	Group D: hCG trigger (n = 141)	
Total dose of FSH (in IU)	1679 (554)	1612 (525)	0.54	1901 (602)	1923 (701)	0.78
Duration of FSH (in days)	9.85 (2.44)	9.40 (1.34)	0.12	9.42 (1.76)	9.22 (1.87)	0.37
Follicles ≥11 mm	16.6 (3.9)	17.1 (3.1)	0.66	8.1 (2.9)	7.7 (3.0)	0.35
Oocytes, n[a]	13.7 (5.9)	13.5 (5.7)	0.93	6 (3–9)	3 (1–6)	0.21
Metaphase II, n[a]	10 (8–16)	10 (7–12)	0.34	5 (3–8)	6 (3–8)	0.30
2 pronuclei oocytes total (IVF & ICSI)[a]	7 (4–9)	7 (4–9)	0.51	3 (2–5)	3 (1–6)	0.77
Transferable embryos[a]	3 (1–5)	3 (1–6)	0.88	3 (2–5)	1 (1–3)	0.51

Values are presented as means (SD).
[a]Skewed values are presented as medians (IQR).

Serum hormone levels, groups at risk of OHSS, A and B (>14 follicles)

Serum hormone levels are presented in Table IV. E_2, FSH, LH and progesterone levels did not differ significantly between groups during the follicular phase—S1 and the day of trigger. In contrast, significant differences were seen on the day of oocyte retrieval and oocyte retrieval +7 (Table IV).

Serum hormone levels, OHSS low-risk groups C and D (≤14 follicles)

Serum E_2, FSH, LH and progesterone levels did not differ significantly between groups during the follicular phase. In contrast, significant differences between groups were seen on the day of oocyte retrieval and oocyte retrieval +7 (Table IV).

Discussion

This study is to our knowledge the largest RCT exploring the effect of an individualized luteal phase hCG support after GnRHa trigger according to the number of follicles on the day of triggering final oocyte maturation. An upper limit of more than a total of 25 follicles of ≥11 mm was set as the exclusion criterion from the study to prevent possible randomization to conventional hCG trigger. Using this concept, no OHSS was seen in the group at risk of OHSS after GnRHa trigger despite supplementation with a bolus of 1.500 IU hCG, compared with an OHSS incidence of 3.4% in the group at risk of OHSS triggered with hCG. In contrast, two cases of late-onset OHSS were seen in the OHSS low-risk group triggered with GnRHa followed by two boluses of 1.500 IU hCG, compared with no OHSS case after hCG trigger.

Based on the results of previous studies (Andersen *et al.*, 2006; Humaidan, 2009; Humaidan *et al.*, 2010, 2011b), in the present study the luteal

Table III Reproductive outcome for women in the two RCTs.

	>14 follicles			≤14 follicles		
	Group A: GnRHa trigger + 1.500 IU hCG	Group B: hCG trigger	RR 95% CI P-value	Group C: GnRHa trigger + 1.500 IU hCG × 2	Group D: hCG trigger	RR 95% CI P-value
Patients, n	60	58		125	141	
Rate of transfer, n (%)	52/60 (86.7)	57/58 (98.3)	0.88 [0.79–0.97] 0.02	110/125 (88.0)	116/141 (82.3)	1.06 [0.96–1.18] 0.18
Embryos transferred, mean (SD)	1.19 (0.40)	1.19 (0.40)	0.99	1.27 (0.45)	1.25 (0.43)	0.98
Positive hCG per embryo transfer, n (%)	25/52 (48.1)	21/57 (36.8)	1.30 [0.83–2.03] 0.23	47/110 (42.7)	41/116 (35.3)	1.20 [0.87–1.67] 0.25
Clinical pregnancy per patient, n (%)	21/60 (35.0)	17/58 (29.3)	1.19 [0.70–2.02] 0.51	43/125 (34.4)	40/141 (28.4)	1.21 [0.84–1.73] 0.28
Ongoing pregnancy per patient, n (%)	17/60 (28.3)	15/58 (25.9)	1.09 [0.60–1.98] 0.76	37/125 (29.6)	36/141 (25.5)	1.15 [0.78–1.71] 0.45
Implantation rate, n (%)	22/62 (35.5)	20/68 (29.4)	1.20 [0.73–1.98] 0.46	49/138 (35.5)	43/145 (29.7)	1.19 [0.85–1.67] 0.29
Early pregnancy loss, n (% of positive hCG)	4/25 (16.0)	4/21 (19.0)	0.84 [0.23–2.95] 0.78	4/47 (8.5)	1/41 (2.4)	3.48 [0.40–29.98] 0.25
OHSS rate, n (%)	0/60 (0)	2/58 (3.4)	0.24	2/125 (1.6)	0/141 (0)	0.22

RR, relative risk; CI, confidence interval; OHSS, ovarian hyperstimulation syndrome.

phase support after GnRHa trigger was individualized according to the ovarian response on the day of triggering final oocyte maturation. As a threshold of >14 follicles ≥11 mm on the day of ovulation induction previously has been shown to predict 87% of severe OHSS cases (Papanikolaou et al., 2006), this cut-off level was used to create two risk groups: a group at risk of OHSS and an OHSS low-risk group. The group at risk of OHSS was randomized to receive either 5.000 IU hCG or GnRHa trigger followed by one bolus of 1.500 IU hCG on the day of oocyte retrieval. In contrast, the OHSS low-risk group was randomized to receive trigger with either 5.000 IU hCG or GnRHa trigger and two boluses of 1.500 IU hCG—one bolus on the day of oocyte retrieval and an additional bolus of 1.500 IU hCG on the day of oocyte retrieval +5.

In the group at risk of OHSS no OHSS case occurred after GnRHa trigger supplemented with one bolus of 1.500 IU hCG, whereas an incidence of 3.4% was seen in the hCG-triggered group. The apparent reduction in the OHSS rate in the OHSS risk group despite supplementation with one bolus of 1.500 IU hCG is in line with the results of a recent retrospective analysis in 71 high-risk patients using the same protocol (Radesic and Tremellen, 2011). However, in their study no upper limit regarding the number of follicles on the day of trigger were set, which might explain that one patient (1.4%) developed a severe late-onset OHSS (Radesic and Tremellen, 2011).

Our decision not to include patients with more than a total of 25 follicles of ≥11 mm in the present study, apart from avoiding randomization to hCG trigger, was supported by the results of a previous pilot study including 12 patients with ≥25 follicles of >11 mm (Humaidan, 2009). In that study patients received GnRHa trigger and one bolus of 1.500 IU hCG (Humaidan et al., 2006) and a high live birth rate (6/12) was obtained, however at the cost of one late-onset moderate OHSS case.

The 3.4% OHSS incidence in the group at risk of OHSS triggered with hCG (5.000 IU) correlates well with an incidence of 2.1% severe OHSS in an overall IVF population triggered with hCG (Papanikolaou et al., 2006) and the 2% incidence seen in our previous study in patients triggered with hCG (Humaidan et al., 2010). Taken together, GnRHa triggering followed by supplementation with one bolus of 1.500 IU hCG appears to reduce the OHSS incidence in the group at risk of OHSS when an upper limit of 25 follicles is used as a cut-off. Above this limit, to completely eliminate OHSS we recommend either an intensive luteal phase support strategy with E_2 and progesterone (Kummer et al., 2011; Benadiva and Engmann, 2012) or, even better, a freeze-all policy (Devroey et al., 2011).

Regarding the OHSS low-risk groups, two patients (1.4%) developed late-onset OHSS after GnRHa trigger supplemented with two boluses of 1.500 IU hCG, whereas no OHSS was seen after trigger with 5.000 IU hCG: the patients had 11 and 13 follicles, respectively, on the day of trigger and, thus, were close to the threshold set to distinguish OHSS low-risk patients from patients at risk of OHSS. Both patients were treated on an outpatient basis. Thus, supplementation with two boluses of 1.500 IU hCG in a group of patients considered to be at low risk of OHSS induced two cases of late-onset OHSS. This finding necessitates future studies to determine the minimal hCG activity needed to support the luteal phase after GnRHa trigger in this specific population.

Importantly, the present trial was not powered to assess the reproductive outcome, and observations regarding this issue can be a chance finding. However, in the present sample size the reproductive outcome did not differ between GnRHa-triggered and hCG-triggered

Table IV Endocrine parameters for women in RCT of GnRHa trigger and individualized luteal phase hCG support according to ovarian response to stimulation.

	>14 follicles		P-value A versus B	≤14 follicles		P-value C versus D
	Group A: GnRHa trigger + 1500 hCG (n = 60)	Group B: hCG trigger (n = 58)		Group C: GnRHa trigger + 1500 hCG × 2 (n = 125)	Group D: hCG trigger (n = 141)	
E2, S1 (pmol/ml)[a]	155 (133–174)	157 (130–179)	0.85	167 (137)	162 (133–201)	0.60
E2, day of triggering (pmol/ml)[a]	7936 (4304–10 583)	6416 (3313–8910)	0.10	3625 (2472–5436)	3685 (2349–5675)	0.89
E2, day of OR + 7 (pmol/ml)[a]	3082 (1198–5032)	3262 (1958–6006)	0.32	3405 (2365–4607)	2076 (1348–3581)	<0.0001
LH, day of OR (IU/l)[a]	2.5 (1.8–2.9)	0.7 (0.4–1.1)	<0.0001	2.6 (1.9–3.7)	1.1 (0.6–2.2)	<0.0001
LH, day of OR + 7 (IU/l)[a]	0.1 (0.1–0.5)	0.2 (0.1–0.5)	0.48	0.1 (0.1–0.1)	0.3 (0.1–0.7)	<0.0001
FSH, S1 (IU/l)	5.6 (1.7)	5.8 (1.4)	0.66	6.9 (1.7)	7.0 (2.1)	0.93
FSH, day of OR (IU/l)	8.0 (2.4)	5.5 (1.9)	<0.0001	10.6 (3.5)	7.4 (2.8)	<0.0001
FSH, day of OR + 7 (IU/l)[a]	0.5 (0.2)	0.4 (0.2)	0.001	0.6 (0.5–0.9)	0.6 (0.5–0.8)	0.91
Progesterone, day of triggering (nmol/l)	3.1 (1.6)	3.3 (1.2)	0.28	2.8 (1.6)	2.7 (1.5)	0.98
Progesterone, day of OR (nmol/l)[a]	28.9 (11.3)	43.8 (21.3)	<0.0001	14.3 (9.9–21.6)	23.0 (14.8–30.2)	<0.0001
Progesterone, day of OR + 7 (nmol/l)[a]	77.4 (50.3–102.9)	84.5 (56.6–129)	0.15	409 (238–574)	75.3 (41.2–122.8)	<0.0001
hCG, day of OR + 7 (IU/l)[a]	2.5 (0.7)	2.9 (0.9)	0.008	26 (15–39)	3 (2–4)	<0.0001

Values are presented as means (SD).
E2, estradiol; S1, stimulation Day 1; OR, oocyte retrieval.
[a]Skewed values are presented as medians (IQR).

groups. This is interesting as a previous meta-analysis of GnRHa trigger and modified luteal support showed a non-significant difference of 6% in the live birth rate, however in favour of hCG trigger (Humaidan et al., 2011a). Furthermore, in a previous randomized study we reported a non-significant difference of 7% in delivery rates between GnRHa trigger and hCG trigger (10.000 IU)—in favour of hCG trigger (Humaidan et al., 2010). In that study a mean of 9 oocytes was retrieved in both groups and approximately one-third of patients in each study group had at least 14 follicles ≥11 mm on the day of trigger. Furthermore, as the positive hCG rate per embryo transfer was 48% for both groups, the difference in ongoing pregnancy and delivery rates seemed to be caused mainly by more early pregnancy losses in the GnRHa trigger study group. When scrutinizing the data, a higher early pregnancy loss was seen in patients with 14 or less follicles ≥11 mm when compared with patients with more follicles on the day of trigger. This prompted us to suggest the current modification of our previous luteal phase support protocol taking into account the number of follicles on the day of triggering final oocyte maturation, when deciding the total dose of hCG administered.

As patients with 14 or less follicles ≥11 mm are less likely to develop OHSS (Papanikolaou et al., 2006), we decided to add an additional bolus of 1.500 IU hCG in this group on the expected day of implantation—Day 5 after oocyte retrieval (Tesarik et al., 2006)—to support early implantation in terms of increasing the endogenous progesterone production and up-regulating LH activity-based pregnancy-promoting actions. This modification in the subgroup of patients with 14 or less follicles resulted in implantation, and clinical and ongoing clinical pregnancy rates comparable to those of hCG trigger, however, at the cost of two OHSS cases. Moreover, the same pattern regarding reproductive outcome was seen in patients with >14 follicles who received GnRHa trigger. Taken together, by individualizing the modified luteal phase support after GnRHa trigger according to number of follicles on the day of trigger, we obtained a reproductive outcome after GnRHa trigger similar to that seen after hCG trigger.

In the present study we explored the concept of a modified luteal phase support after GnRHa trigger, using a bolus of hCG to compensate for the LH activity deficiency during the early luteal phase seen after GnRHa trigger and, thus, dissociating the ovulation trigger from the luteal support (Humaidan et al., 2006, 2010; Humaidan, 2009). In a previous uncontrolled retrospective study, Castillo et al. (2010) used repeated luteal boluses of 250–1000 IU hCG after GnRHa trigger in 192 OHSS risk patients with 15 or more follicles ≥14 mm on the day of ovulation trigger. In addition, micronized progesterone 600 mg was administered vaginally on a daily basis. The authors reported a pregnancy rate of 43% and a total OHSS rate (moderate and severe) of 8% and concluded that 250–500 IU hCG every third day after GnRHa trigger seemed to reduce the OHSS rate in OHSS risk patients as well as to secure the reproductive outcome.

To avoid the long-acting luteotropic actions of hCG, Papanikolaou et al. (2011), in a small randomized proof of concept study in an OHSS low-risk group of patients having either hCG or GnRHa trigger, supplemented GnRHa-triggered patients with a total of six doses of 300 IU recombinant LH every other day during the luteal phase. Both hCG-triggered and GnRHa-triggered patients received daily luteal phase support in the form of micronized progesterone 600 mg. No OHSS was reported with either protocol and no difference was seen between groups regarding the reproductive outcome.

Another approach is the 'dual trigger' strategy, combining the triggering benefits of a bolus of GnRHa with the long-acting LH activity of a small bolus of hCG administered at the same time as the GnRHa bolus (Shapiro et al., 2008). Recently, Shapiro et al. (2011) retrospectively reported the effect of dual trigger in a group of high-risk OHSS patients with a mean of 28 follicles on the day of trigger. A total of 182 patients received a bolus of GnRHa combined with a mean of 1.428 IU hCG, resulting in one late-onset OHSS case in 182 patients (0.5%) and an ongoing pregnancy rate of 58%.

Finally, intensive luteal phase support with E_2 and progesterone only in OHSS high-risk patients triggered with a bolus of GnRHa was originally described by Babayof et al. (2006). No OHSS case was reported, however, at the cost of a disappointingly low reproductive outcome. Later Engmann et al. (2008) used a similar approach in 33 OHSS high-risk patients, showing the prevention of OHSS as well as good ongoing pregnancy rates; a finding which was recently supported by Imbar et al. (2012) in an observational trial in OHSS high-risk patients. These reports, however, have been contrasted by the findings of others (Orvieto et al., 2006; Orvieto, 2012), who used the same intensive luteal phase protocol with E_2 and progesterone as Engmann et al. (2008) and, nevertheless, reported low ongoing pregnancy rates. Recently, it was suggested that patients need to be stratified according to the E_2 level on the day of trigger if intensive luteal phase support with E_2 and progesterone only is to be used. Thus, OHSS risk patients with E_2 levels >4.000 pg/ml may be supplemented during the luteal phase with intensive E_2 and progesterone only, whereas patients with E_2 levels <4.000 pg/ml should receive a dual trigger (GnRHa + 1.000 IU hCG) as well as intensive luteal phase support with E_2 and progesterone (Griffin et al., 2012).

A limitation of the present study is that it had to be discontinued before the estimated sample size had been achieved due to unforeseen events, as described above. Thus, we did not reach the sample size necessary to reach statistical significance regarding the primary end-point, i.e. OHSS incidence in the groups at risk of OHSS. Moreover, the OHSS incidence in the OHSS risk group triggered with hCG was primarily estimated to be 12% based on a previous publication (Lainas et al., 2007), but turned out to be lower, which might be due to the presence of a higher total number of follicles on the day of trigger in the Lainas et al. (2007) study. However, the strength of the present study is that it is the largest RCT until now exploring modified luteal phase support after GnRHa trigger; thus, the number of cycles randomized in the present study approaches the total number of cycles included (N: 476 from six studies) in the most recent meta-analysis on GnRHa trigger followed by modified luteal phase support (Humaidan et al., 2011a). As such, the current data could be of importance for future meta-analyses.

Other limitations of the study are the lack of blinding of patients, nurses and physicians and, moreover, the fact that, for women with <14 follicles ≥11 mm, no power calculation was performed due to the novelty of the protocol used (dual hCG 1.500 IU administration). Therefore, no safe conclusions can be drawn for this population. Nonetheless, the occurrence of two OHSS cases in this population after the administration of two boluses of 1.500 IU hCG following GnRHa trigger indicates that this practice should not be recommended. Currently we are investigating the minimal hCG activity needed for this population to avoid OHSS.

In conclusion, this large RCT in patients at risk of OHSS (15–25 follicles) and OHSS low-risk patients (≤14 follicles) explored the effect of an individualized modified luteal phase support after GnRHa trigger on the

incidence of OHSS. No OHSS was seen in the high-risk group triggered with GnRHa followed by one bolus of hCG; in contrast, two cases of OHSS in the OHSS low-risk group triggered with GnRHa were seen, most probably due to the amount of hCG administered as a second bolus. Interestingly, the reproductive outcome was similar to that of hCG trigger for both high- and low-risk groups triggered with GnRHa. Future trials should focus on the minimal hCG activity needed for luteal phase support in the OHSS low-risk group to secure the reproductive outcome in the absence of OHSS.

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Authors' roles

All authors actively participated in planning of the study design, manuscript drafting and acceptance of the final draft. Moreover, statistical analyses were performed by N.P.P.

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Conflict of interest

None declared.

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